Protocol of constructing Histag-CsgA-SpyCatcher-Histag

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1. Target sequence for Gibson assembly into pET22b(+)

ACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACC
GCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATA
CGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATAT
ACATATGAAACTTTTAAAAGTAGCAGCAATTGCAGCAATCGTATTCTCCGGTAGCGCTCTGGCAGGTGTTGTTCCTCA
GTACGGCGGCGGCGGTAACCACGGTGGTGGCGGTAATAATAGCGGCCCAAATTCTGAGCTGAACATTTACCAGTAC
GGTGGCGGTAACTCTGCACTTGCTCTGCAAACTGATGCCCGTAACTCTGACTTGACTATTACCCAGCATGGCGGCGGT
AATGGTGCAGATGTTGGTCAGGGCTCAGATGACAGCTCAATCGATCTGACCCAACGTGGCTTCGGTAACAGCGCTAC
TCTTGATCAGTGGAACGGCAAAAATTCTGAAATGACGGTTAAACAGTTCGGTGGTGGCAACGGTGCTGCAGTTGAC
CAGACTGCATCTAACTCCTCCGTCAACGTGACTCAGGTTGGCTTTGGTAACAACGCGACCGCTCATCAGGGTGGCG
GTGGCTCTGGTGGCGGTGGCTCTGTTGATACCTTATCAGGTTTATCAAGTGAGCAAGGTCAGTCCGGTGATATGACA
ATTGAAGAAGATAGTGCTACCCATATTAAATTCTCAAAACGTGATGAGGACGGCAAAGAGTTAGCTGGTGCAACTAT
GGAGTTGCGTGATTCATCTGGTAAAACTATTAGTACATGGATTTCAGATGGACAAGTGAAAGATTTCTACCTGTATCCA
GGAAAATATACATTTGTCGAAACCGCAGCACCAGACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAATGAG
CAAGGTCAGGTTACTGTAAATGGCAAAGCAACTAAAGGTGACGCTCATATTCTCGAGCACCACCACCACCACCACTG
AGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCC
CTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT

Red: CsgA
GGTGGCGGTGGCTCTGGTGGCGGTGGCTCT: linker
Green: SpyCatcher

First

PCR to get CsgA and SpyCatcher with the flanking sequence for Gibson assembly with each other and the backbone plasmid. PCR progression: 98(1:00)-**98(0:10)-58/60(0:30)-72(1:00)**-72(5:00). The bold sequence for 34x.

F1: AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAAACTTTTAAAAGTAGC
R1: ATC AAC AGA GCC ACC GCC ACC AGA GCC ACC GCC ACC CTG ATG AGC GGT CGC GTT GT
F2 : GCTCATCAGGGTGGCGGTGGCTCTGGTGGCGGTGGCTCTGTTGATACCTTATCAGGTTT
R2: CGG ATC TCA GTG GTG GTG GTG GTG GTG CTC GAG AAT ATG AGC GTC ACC TTT AGT TGC TT

PCR protocol	
template	0.5
F	1
R	1
ddH2O	20
Q5(NEB) 2xMix	22.5

Gibson protocol	
CsgA	1
SpyCatcher	1
Restricted Backbone	3
Gibson 2x Mix	5
ddH2O	0

Second

We used Easy Pure Quick Gel Extraction Kit to purify the sequences for Gibson Assembly. The Gibson Assembly protocol should refer to the specific product instruction.

Then

we use the following primers to amplify the target sequence with flanking sequence for endonuclease KpnI, MluI. The system is as the same above. Then ligation into the backbone

pZA CmR rr12 pL(tetO), right after the promoter of atc. The plasmid is optimized for CsgA secretion. It is obtained from Zhong Lab, the lab of one of our PI.
F: CGGGGTACC ATGAAACTTTTAAAAGTAGC
R: ATATACG CGT TCA GTG GTG GTG GTG GTG GTG